SEROLOGICAL STUDIES ON *CYSTICERCUS FASCIOLARIS*

(CESTODA: TAENIIDAE)

A THESIS

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CHAPTER I

INTRODUCTION

Antibodies are specific proteins produced by an animal in response to the introduction of a foreign antigen. This antigen may be a component of a virus, bacterium, protozoan, and metazoan parasite or the total organism. Certain specific cells of the animal such as the T-cells of the thymus and the tissues of the reticuloendothelial system may produce the antibodies, then release them into the blood stream. The component of blood that may be examined for antibodies is known as serum which is separated from clotted blood. The serum containing the antibodies is known as an immune serum or antiserum which is capable of reacting with the homologous antigen either in vivo or in vitro. The in vitro study of antigen-antibody reactions is known as serology. Some serological tests for parasitic diseases include agglutination, precipitation, complement fixation, circumlarval precipitation, fluorescent antibody methods, and nonspecific chemical flocculation methods. Serological tests

to demonstrate circulating antibodies in specific cestode infections have long attracted the attention of many investigators (Table 1).

Among numerous cestode infections, many of the earlier studies have been concerned with Cysticercus fasciolaris Rud. 1808, C. fasciolaris, the larval form of the cat tapeworm Hydatigera taeniaeformis (= Taenia taeniaeformis), develops in the liver of rats and mice. In the very early stages of development in the intermediate host, the cysticercus is a bladder containing a single inverted scolex. Later, the scolex evaginates, segments into a young tapeworm, and retains the bladder at its terminal end. This type of larva is known as a strobilocercus. Wilhelmi (1940) conducted precipitin ring tests to determine species specificity and interrelationships of Cysticercus fasciolaris with Hydatigera taeniaeformis. In these tests, lyophilized worm material was delipidized using an ether-alcohol mixture and suspended in physiological saline. Antisera were obtained from rabbits immunized with the lipid-free extracts of C. fasciolaris and H. taeniaeformis. More recently, Leid and Williams (1974) used the agar-gel precipitin test to demonstrate precipitating antibodies against C. fasciolaris in immune serum from infected rats.

Table 1.--Some reported serological reactions with cestodes

Cestode	Antigen(s)	Serological Test	Author(s)
Echinococcus granulosus	scolex antigen whole cyst fluid	Bentonite flocculation	Norman, Sadun and Allain (1959)
	hydatid fluid cyst membrane scolex antigen	Agar double diffusion	Kagan and Norman (1961)
Cysticercus cellulosae	alcoholic extract *C. cellulosae*	Complement fixation	Nieto (1956)
	acetone extract *C. cellulosae*	Precipitin ring	Biagi (1958)
Hymenolepis nana var. fraterna	saline extract	Precipitin ring Complement fixation Direct Agglutination	Larsh (1943)
	egg, cysticercoid, and adult stages	In vitro precipitin	Heyneman and Welsh (1959)
Taenia saginata and *T. solium*	procine antigen of lyophilized cysticerci suspended in saline	Hemagglutination Agar double diffusion Precipitin ring	Proctor, Powell, Eldson-Dew (1966)

The present investigation was undertaken to examine several types of homologous serological responses of antibodies produced in rabbits to C. fasciolaris antigen. The serological tests employed consisted of the micro-precipitin test, agar-gel precipitin test, immunoelectrophoresis test, and the indirect hemagglutination test. Agar-gel precipitin tests were performed with C. fasciolaris antigen and antiserum against Taenia crassiceps, T. saginata, and Echinococcus granulosus to determine if cross reactions could be obtained.

CHAPTER II

REVIEW OF LITERATURE

Miller and Dawley (1928) performed precipitin tests on serum from a rat which was heavily infected with Cysticercus fasciolaris. Two series of tubes were set up with diluted antigen and diluted antisera. Precipitin formation could not be demonstrated by their methods.

Miller (1931a) demonstrated acquired immunity to superinfection with C. fasciolaris in the albino rat. Rats with a few to many days old cysts were protected against infections when fed a challenge dose of onchospheres. This protection was greater in naturally acquired immunity than in the case of artificially immunized rats.

Miller (1931b) demonstrated protective antibodies in albino rats which were artificially immunized with fresh or powdered worm material of the larvae and adults of Taenia taeniaeformis. When these rats were experimentally infected with onchospheres, more larvae were observed in the controls than in the immunized rats.

Miller and Gardiner (1932) observed that sera from infected rats were more effective than sera from actively immunized rats in preventing the development of cysts. Immune sera were effective in arresting the development of C. fasciolaris when administered within nine days after rats received a challenge dose of onchospheres. In rats which were given the same amount of immune sera ten days or more after infection, cyst development was practically equal to that of the controls.

A high level of immunity long after the removal or loss of Cysticercus fasciolaris has been reported by Miller and Massie (1932). The development of cysticerci was completely inhibited in albino rats from which the worms of an initial infection had been removed. The control rats were infected with an average number of seventy-seven larvae.

Miller and Gardiner (1934) conducted experiments to determine the effectiveness of immune serum from lightly and heavily infected rats. They observed that a direct relationship existed between the degree of infection in the donor and the protective power of the serum. Immune serum was also capable of conferring passive immunity within ten days after infection of the donor rat.

Miller (1935a) used antigens in the form of powdered

Diphyllobothrium latum, Taenia saginata, Hymenolepis sp. and Dipylidium sp. to immunize albino rats followed by a challenge dose of onchospheres. It was observed that little or no protection was established in these rats but the introduction of living T. pisiformis into the peritoneal cavity resulted in a high level of protection against C. fasciolaris infection.

Miller (1935b) reported that protective immunity may be passively transferred from female rats infected with C. fasciolaris to their offspring. The young of immune and control rats were infected at various intervals with onchospheres. A higher degree of protection was conferred upon those offspring from infected females than those from actively immunized females. This passive immunity was found to persist beyond six weeks after birth.

Campbell (1936) artificially immunized albino rats with polysaccharide and protein fractions of worm material. He observed that albuminoids and the polysaccharide fractions produced a significant degree of resistance while, albumins and metaproteins provided little if any protection against challenge doses of onchospheres.

In a series of experiments, Campbell (1938, a, b, c) demonstrated the presence of humoral protective antibodies

which could establish passive immunity in rats. Campbell (1938a) reported that rats which received sera from other rats which were infected for fourteen, twenty-one, and twenty-eight days were completely protected against a challenge infection. Immune serum from rats with a light infection induced little if any passive immunity. Rats which received sera from other rats that were infected for fifteen and twenty days were protected to some degree. He concluded that the degree of infection up to the twenty-eighth day influenced the production of humoral antibodies and that the antibody response was greater in heavily infected than in lightly infected animals. However, after twenty-eight days both sera from heavily and lightly infected rats had approximately the same degree of protection.

Campbell (1938b) artificially immunized and infected rats and rabbits with Cysticercus fasciolaris and C. pisiformis respectively to determine if antibodies could be removed by absorption with whole worm material. He observed that protective antibodies were completely absorbed from the serum of artificially immunized and infected animals when obtained after a two week period but, the protective property was not removed from the serum of infected animals obtained four weeks after the infection. It was suggested

that an early and late immunity was found during the course of infection which was stimulated by antigens released from growing larvae. Campbell (1938c) observed that absorption reduced the prophylactic power of immune serum to destroy the parasites before encystment.

Wilhelmi (1940) used the precipitin ring test to determine species specificity and interrelationships of organisms. Cysticercus fasciolaris and Hydatigera taeniaeformis antigens were lyophilized, delipidized, and suspended in physiological saline. Rabbits were immunized with lipid-free extracts of C. fasciolaris and H. taeniaeformis. Precipitin ring tests were positive for the homologous and heterologous reaction of C. fasciolaris and H. taeniaeformis.

Greenfield (1941) reported that rats of at least twenty-five days old were more susceptible to C. fasciolaris, whereas rats infected on the day of birth showed no indication of cysts development in the liver.

Chen (1950) reported that immune serum from rats infected four weeks or less with C. fasciolaris did not cause precipitates to form around onchospheres, although it completely protected rats from heavy infections. Both normal rat serum and immune serum produced similar results in that they caused the bladder wall to collapse while

slight precipitates formed. This cysticercoidal property was lost after inactivation for thirty minutes at 56 C and after storage in the cold for one week.

Kraut (1956) studied electrophoretic patterns of sera from rats experimentally infected; artificially immunized; and passively immunized with immune sera to determine if Cysticercus fasciolaris influenced protein metabolism and the host's humoral response to the parasite. Investigations were also made to determine which protein fractions contained antibodies that appeared during the course of the infection. He observed that an increase in albumin was indicative of impaired liver function; an increase in beta-globulin was due to the beginning of the proliferative stage; whereas, the increase in alpha 2-globulin was associated with the proliferative activity of larvae. He reported that there was no evidence that the protein component containing the early immunity factor destroyed larvae prior to encystment. It was suggested that electrophoretic studies of serum proteins served as indicators of the physiological state of the animal and that changes in serum proteins were non-specific responses correlated with liver involvement.

Oliver (1961) reported wide differences in

susceptibility of rats, mice, guinea pigs, and hamsters to C. fasciolaris infections. He observed that hamsters were unsuitable hosts and there was no evidence that worms could reach the liver. The parasites reached the liver in guinea pigs and some mouse strains but failed to develop into infective cysts. Mouse and rat strains were susceptible in that the larvae were able to develop into infective cysts. Male mice were more susceptible than female mice.

Olivier (1962) studied the effect of cortisone on the natural resistance to Cysticercus fasciolaris in mice. He demonstrated that cortisone increased the average size of the parasite in mice and that the time and duration of cortisone administration was very critical.

Using the agar-gel precipitin technique, Leid and Williams (1974) observed the presence of precipitating antibodies in rats infected with C. fasciolaris.

CHAPTER III

MATERIALS AND METHODS

Male and female albino rats four to eight weeks of age of the Sprague-Dawley strain were infected with Hydatigera taeniaeformis onchospheres by means of a stomach tube attached to a 3 cc syringe. Cats were used for maintaining a source of H. taeniaeformis eggs. Gravid proglottids were obtained from fecal droppings from the cats and were washed in physiological saline. The segments were macerated in a glass tissue homogenizer in order to free the onchospheres. The macerated material was diluted with 0.9 percent saline and a uniform suspension was fed to the rats. Rats were killed at intervals varying from six to ten weeks after infection. The cysts were dissected from infected livers, washed in 0.9 percent physiological saline, lyophilized, and stored in the freezer until ready for use.

The antigen for animal inoculations was prepared by suspending lyophilized Cysticercus fasciolaris worms in 1 ml of 0.9 percent physiological saline and 1 ml of Freund's

complete adjuvant (Cappel Laboratories). Three rabbits received subcutaneous injections of antigen during a two-week period on days one and four of each week. Rabbit A received increasing amounts of the antigen which consisted of 5, 10, 15, and 20 mg whereas, rabbit B received 10, 20, 30, and 40 mg. Both rabbits were bled four and six weeks after the last antigen injection. Each rabbit received a 10 mg booster injection during the ninth week and was bled twelve days later. Rabbit C received 10, 20, 30, and 40 mg of the antigen and was bled two weeks after the last injection. Rabbit D served as the control rabbit and was injected with 1 ml of Freund's complete adjuvant as in the experimental animals.

The rabbits were bled from the marginal ear vein with a twenty gauge needle. The clotted blood was ringed and centrifuged. The sera were collected and stored in the deep freezer until ready for use. Before use, the sera were inactivated at 56 C for thirty minutes.

A slight modification of the method of Chaffee, Bauman, and Shapilo (1954) which consisted of extraction in borate buffer, pH 7.6, for one week was employed in the preparation of the antigen for the micro-precipitin, agar-gel precipitin, and indirect hemagglutination tests. The micro-precipitin tests were performed as follows: the antigen was

doubly diluted to a final dilution of 1:32 with phosphate-buffered saline, pH 7.2, and was carefully overlaid with undiluted antiserum. In another series of tests, antiserum was doubly diluted to a final dilution of 1:32 with phosphate-buffered saline, pH 7.2, and was carefully overlaid with undiluted antigen. Control tests were carried out using the antigen and phosphate-buffered saline, antiserum and phosphate-buffered saline, and with the antigen and normal serum. The tests were read after incubation at room temperature for one hour.

The agar-gel precipitin test of Ouchterlony (1953) was employed using the slide technique. Slides were pre-coated with a 0.1 percent Noble agar solution and allowed to dry. A 1 percent Noble agar solution was prepared by dissolving 10 g of special Noble agar, 5 g NaCl, 7.5 g glycine, and 1 ml of merthiolate in 1,000 ml of distilled water. After drying, 5 ml of the Noble agar solution was pipetted on to each slide. The agar was allowed to gel after which diagonal and circular templates were cut into it with a Gelman immunodiffusion punch set. The bottom edges of the wells were sealed with a minute drop of agar.

In one series of tests, Cysticercus fasciolaris antigen was doubly diluted to a final dilution of 1:32 and

placed in the center well while undiluted antiserum was placed in wells on either side in the diagonal templates. In another series of tests, antiserum was doubly diluted to a final dilution of 1:32 and placed in the center well and undiluted antigen in the wells on either side. The position of antigen to antiserum was reversed in each series. The controls for this test consisted of normal rabbit serum and the antigen, immune serum and phosphate-buffered saline, and the antigen and phosphate-buffered saline.

Tests were performed in order to determine if cross reactions were possible. C. fasciolaris antigen was tested with rabbit antiserum against Echinococcus granulosus, Taenia saginata, and T. crassiceps. These sera were obtained from the Parasitology Division, Bureau of Laboratories, Center for Disease Control, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia. Circular templates were prepared with C. fasciolaris antigen in the center well and T. crassiceps antiserum in the peripheral wells. Templates were also prepared using antiserum to T. crassiceps in the center well and the antigen of C. fasciolaris in the peripheral wells. Similar tests were carried out with C. fasciolaris antigen and the homologous antiserum. In another test, both Cysticercus

fasciolaris and Taenia crassiceps antisera were placed alternately in the peripheral wells around Cysticercus fasciolaris antigen in the center well. The controls for the heterologous tests consisted of phosphate-buffered saline in the center well and T. crassiceps antiserum in the peripheral wells and T. crassiceps antiserum in the center well with phosphate-buffered saline in the peripheral wells. For the homologous tests, the controls consisted of C. fasciolaris antigen in the center well with normal serum and phosphate-buffered saline in the peripheral wells and normal serum and phosphate-buffered saline in the center well with C. fasciolaris antigen in the peripheral wells. Other controls consisted of C. fasciolaris alternated with phosphate-buffered saline in the peripheral wells around C. fasciolaris antigen in the center well and T. crassiceps alternated with phosphate-buffered saline in the peripheral wells around C. fasciolaris antigen. Both antisera were alternated with normal serum in the peripheral wells around C. fasciolaris antigen in the center well.

The agar-gel precipitin slides were placed in a moist chamber at room temperature for twenty-four hours. The precipitin lines were recorded as they appeared in the agar. Before staining, the wells on the slides were filled

with distilled water, covered with wet filter paper strips, and allowed to dry for a period of two to three days. The filter paper was removed and the excess reactants soaked out with phosphate-buffered saline overnight. The slides were stained with Amido black 10 B and destained with acetic acid according to the method of Crowley (1958).

For immunoelectrophoresis (IEP) tests, the micro-slide technique was applied according to the method of Williams and Grabar (1955). The agar for this test was prepared by dissolving 20 g of special Noble agar in 500 ml of distilled water and 500 ml of veronal buffer pH 8.8. Glass slides for this test were coated as described above for the agar-gel precipitin test. After twenty-four hours, two troughs and one well were punched in the agar of each slide using a Gelman immunoelectrophoresis punch set. The antigen was placed in the center well of each slide.

For each run, a Gelman immunoelectrophoresis chamber was filled with veronal buffer pH 8.8. Microporous wicks were placed at the ends of the agar layer in which one end was allowed to dip in the buffer solution. To determine if sufficient current was passing through the slides, brom phenol blue was placed in the center well of one slide. After the IEP run, the troughs were filled with antiserum

and incubated for twenty-four hours. The staining procedure for the IEP test was similar to the method described by Crowley (1958). The controls for this test consisted of antigen and phosphate-buffered saline, immune serum and phosphate-buffered saline, and normal rabbit serum and antigen.

The indirect hemagglutination test of Boyden (1951) and Stravitsky (1954) was carried out in microtiter plates. Tanned sheep red blood cells were sensitized with an equal volume of the Cysticercus fasciolaris antigen at 37 C for fifteen minutes. The supernatant was decanted and the cells washed twice with 1 percent normal rabbit serum. After incubation for two to three hours, the plates were read for hemagglutination. Controls consisted of a diluent control and a serum control. In the diluent control, sensitized tanned cells were added to wells containing 1 percent normal rabbit serum whereas, the serum control consisted of unsensitized tanned cells which were added to two-fold dilutions of the antiserum.

CHAPTER IV

EXPERIMENTAL RESULTS

The sera of all artificially immunized rabbits were shown to have antibody against Cysticercus fasciolaris when tested by the micro-precipitin, the agar-gel precipitin, and the indirect hemagglutination tests. No reaction occurred in any of the controls. In the agar-gel precipitin tests, a high titered antibody (1:32) was observed in samples of serum from Rabbit A taken four weeks after the last injection. After a booster injection of 10 mg of the antigen, the antibody titer rapidly rose to 1:64.

A positive reaction for the micro-precipitin test was represented by a white precipitate forming at the interphase between antigen and antiserum. The test was positive in sera from rabbits (A and B) collected four to six weeks after the last antigen injection. The highest antibody titer was 1:4 in tests in which antigen was diluted and antiserum undiluted. It is apparent that the highest antibody titer was observed when antiserum was diluted and

antigen undiluted.

In the agar-gel precipitin test, a positive reaction was observed as the formation of two bands of precipitate in diagonal templates in which undiluted antiserum was placed in the center well and undiluted antigen on either side of it (Fig. 1). When the antigen was diluted and the antisera undiluted, the lowest antibody titer for rabbits (A and B) was 1:2 whereas the highest was 1:64. As the procedure was reversed and the antisera were diluted and the antigen undiluted, the lowest antibody titer was 1:2 for rabbits (A and B), whereas, the highest antibody was 1:8. According to these results, it appears that the highest antibody titer was observed when the antigen was diluted and the antisera undiluted.

When undiluted antigen was placed in the center well in the diagonal template and the antiserum doubly diluted on either side, the titer increased in the sera from rabbit A from 1:16 to 1:32 and in rabbit B from 1:2 to 1:4. From these results, it appears that a change in position of antiserum to antigen caused slight variations in the antibody titers.

In the diagonal templates with Taenia crassiceps antiserum on either side of Cysticercus fasciolaris, one

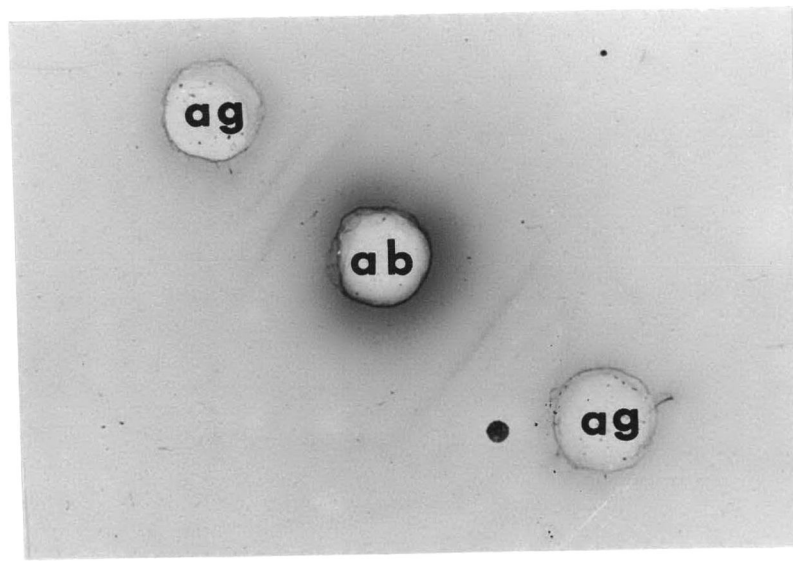

Fig. 1. An agar-gel precipitin slide showing the reaction between Cysticercus fasciolaris antiserum (ab) and C.fasciolaris antigen (ag).

precipitin band was formed (Fig. 2). No bands were observed when C. fasciolaris antigen was tested against Echinococcus granulosus and T. saginata antisera.

In those heterologous tests in which C. fasciolaris antigen was placed in the center well and T. crassiceps antiserum in the peripheral wells, two continuous circular bands were observed. The inner band appeared as a strong precipitate whereas the outer band was relatively weak (Fig. 3). One continuous circular precipitin band was observed when T. crassiceps antiserum was placed in the center well and C. fasciolaris antigen in the peripheral wells (Fig. 4). A similar reaction was observed when Cysticercus fasciolaris antiserum was placed in the peripheral wells and homologous C. fasciolaris antigen in the center well (Fig. 5). No bands appeared in the controls for these tests. The diagonal and circular patterns obtained indicated that C. fasciolaris and Taenia crassiceps share antigens.

To determine further similarities between T. crassiceps and C. fasciolaris, C. fasciolaris antigen was placed in the center well with C. fasciolaris antiserum alternated with T. crassiceps antiserum in the peripheral wells. One continuous band of precipitate was observed in this reaction (Fig. 6). When T. crassiceps antiserum was alternated with

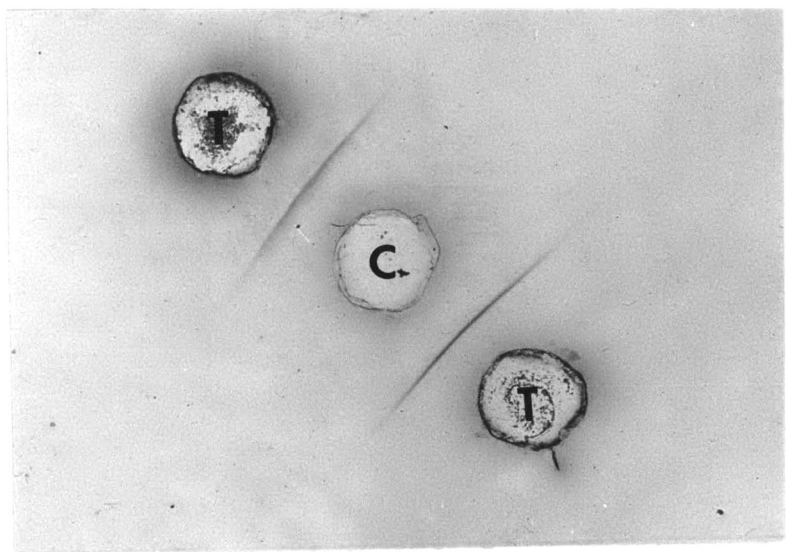

Fig. 2. An agar-gel precipitin slide showing the reaction between Taenia crassiceps antiserum (T) and Cysticercus fasciolaris antigen (C).

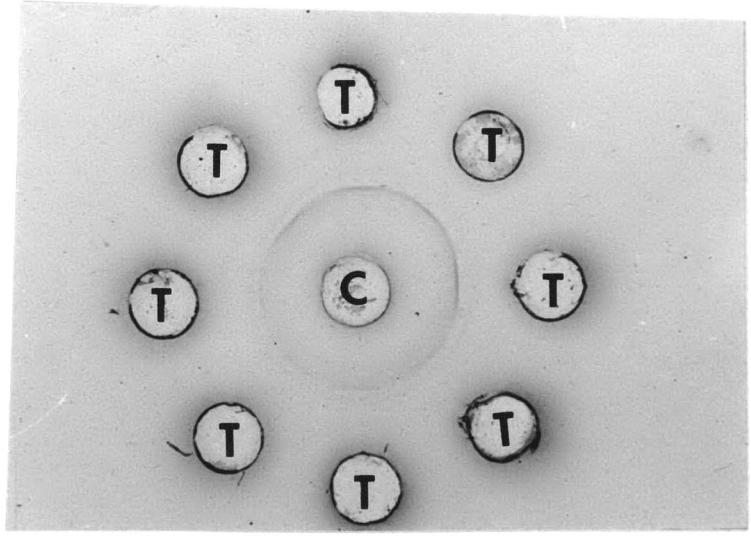

Fig. 3. An agar-gel precipitin slide showing the reaction between Taenia crassiceps antiserum-T (peripheral wells) and Cysticercus fasciolaris antigen-C (center well).

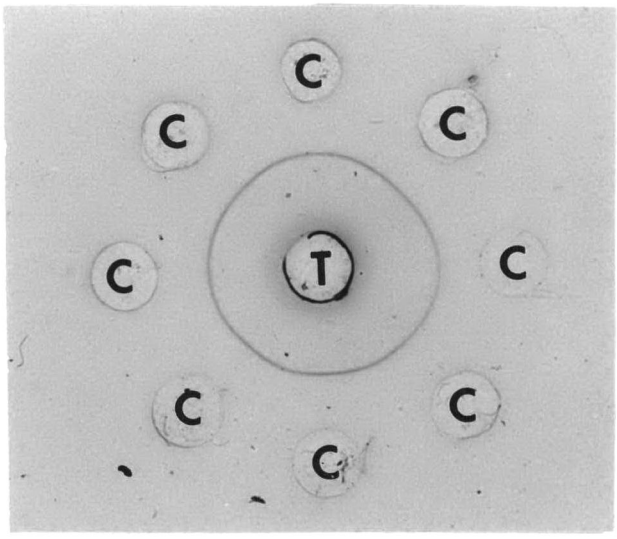

Fig. 4. An agar-gel precipitin slide showing the identity reaction between Taenia crassiceps antiserum-T (center well) and Cysticercus fasciolaris antigen-C (peripheral wells).

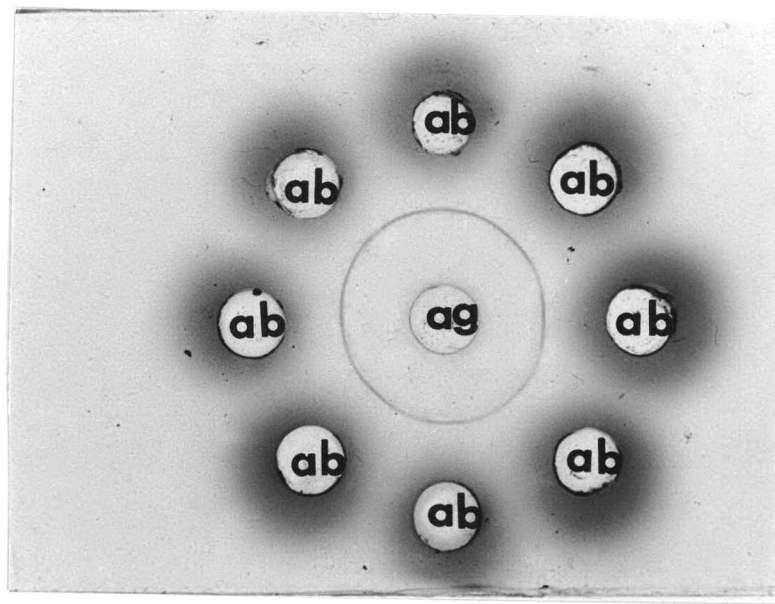

Fig. 5. An agar-gel precipitin slide showing the identity reaction between Cysticercus fasciolaris antiserum (ab) peripheral wells and C. fasciolaris antigen (ag) center well.

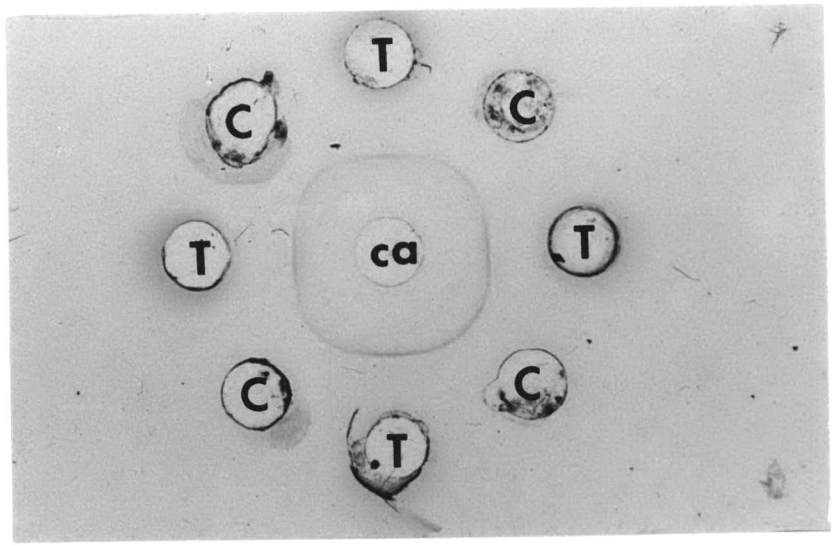

Fig. 6. An agar-gel precipitin slide showing the identity reaction of Cysticercus fasciolaris antiserum (C) alternated with Taenia crassiceps antiserum (T)-(peripheral wells) around C.fasciolaris antigen (ca)-(center well).

phosphate-buffered saline or normal rabbit serum around C. fasciolaris antigen in the center well, a continuous band was not observed. Precipitin bands formed only between T. crassiceps antiserum and C. fasciolaris antigen (Figs. 7 and 8). Similar results were observed when C. fasciolaris antiserum was alternated with phosphate-buffered saline or normal rabbit serum around the homologous C. fasciolaris antigen (Figs. 9 and 10). The results indicated that cross reactions between T. crassiceps and C. fasciolaris could be observed in a system in which the homologous antiserum and heterologous system were alternated around the homologous antigen.

The agar-gel precipitin system for T. crassiceps and C. fasciolaris was examined by the immunoelectrophoresis technique. Three visible arcs appeared between C. fasciolaris antiserum in the troughs and Cysticercus fasciolaris antigen in the center well (Fig. 11). Two arcs appeared at the origin of application of the C. fasciolaris antigen and one arc migrated toward the anode. Four visible precipitin arcs were observed between Taenia crassiceps antiserum in the troughs and C. fasciolaris antigen in the center well (Fig. 12). Two of these arcs appeared near the origin of application of the C. fasciolaris antigen sample and a double arc migrated in the direction of the cathode. The

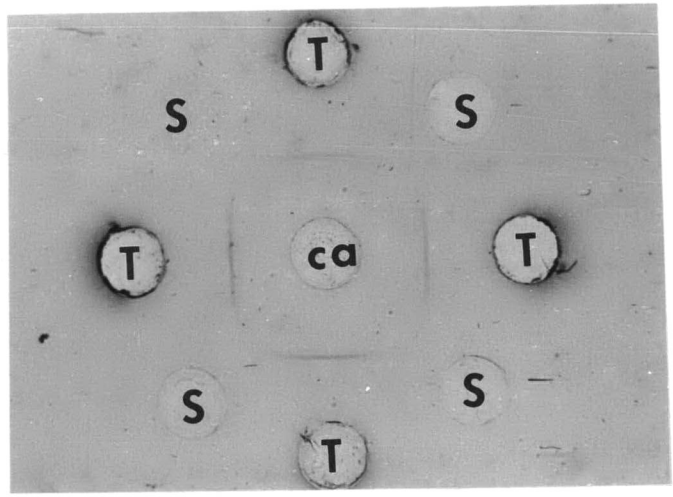

Fig. 7. An agar-gel precipitin slide showing the control reaction of Taenia crassiceps antiserum (T) alternated with phosphate-buffered saline (S) around Cysticercus fasciolaris antigen (ca).

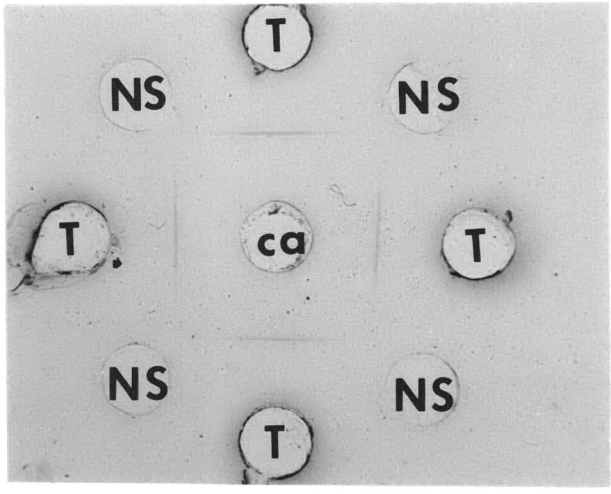

Fig. 8. An agar-gel precipitin slide showing the control reaction of Taenia crassiceps antiserum (T) alternated with normal rabbit serum (NS) around Cysticercus fasciolaris antigen (ca).

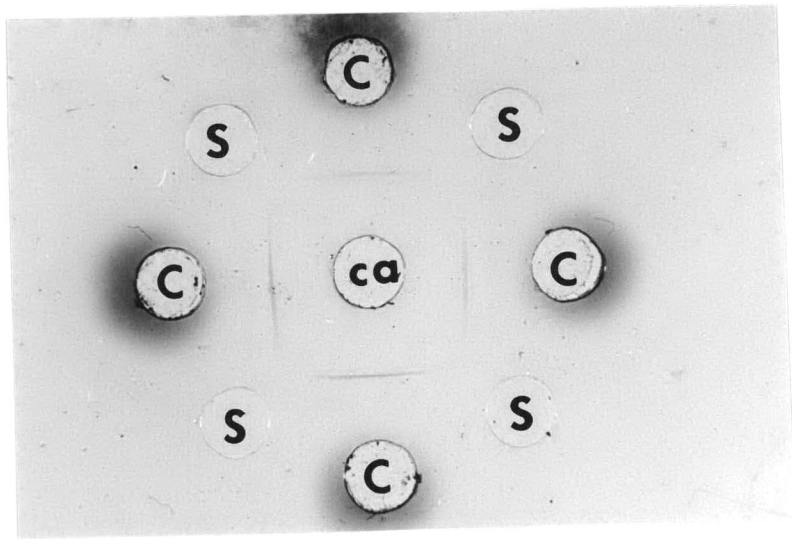

Fig. 9. An agar-gel precipitin slide showing the control reaction of Cysticercus fasciolaris (C) alternated with phosphate-buffered saline (S) around C.fasciolaris antigen (ca).

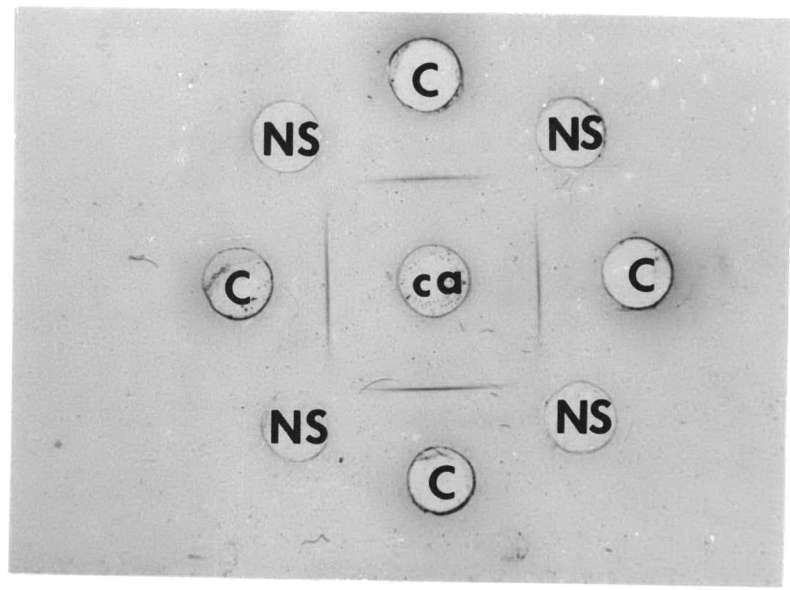

Fig. 10. An agar-gel precipitin slide showing the control reaction of Cysticercus fasciolaris antiserum (C) alternated with normal rabbit serum (NS) around C. fasciolaris antigen (ca).

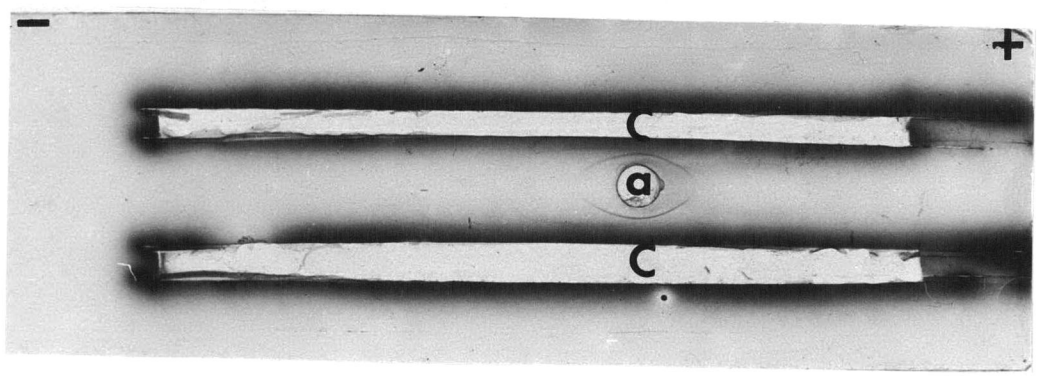

Fig. 11. An immunoelectrophoresis analysis of Cysticercus fasciolaris antiserum (C)- (troughs) and C.fasciolaris antigen (a)-(center well).

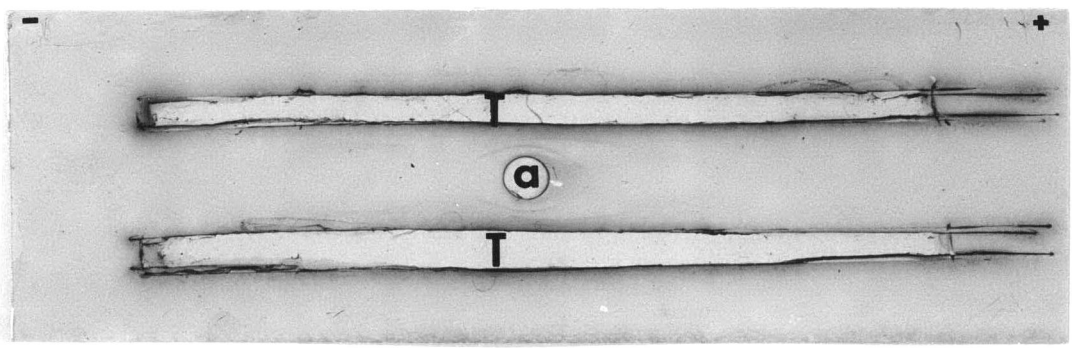

Fig. 12. An immunoelectrophoresis analysis of Taenia crassiceps antiserum (T)- (troughs) and Cysticercus fasciolaris antigen (a)-(center well).

two precipitin arcs near the origin of the C. fasciolaris antigen sample appeared to have been shared by Taenia crassiceps and C. fasciolaris.

CHAPTER V

DISCUSSION

Albino rabbits were successfully immunized with Cysticercus fasciolaris worm material. Precipitating and hemagglutinating antibodies were demonstrated using the micro-precipitin, agar-gel precipitin, and indirect hemagglutination tests. On a comparative basis, the indirect hemagglutination test was the most sensitive; the agar-gel precipitin test was sensitive to a lesser degree; and the micro-precipitin test was the least sensitive. This observation is in accord with that of Proctor, Powell, and Elsdon-Dew (1966) who reported that the hemagglutination test was more sensitive than the agar-gel precipitin test in sera from patients infected with Taenia solium and T. saginata cysticerci and that of Kagan and Bargai (1956) with sera from patients with clinical trichinosis. Rydzewski, Chisholm, and Kagan (1975) also reported that the agar-gel precipitin test was less sensitive than the indirect hemagglutination test and the indirect fluorescent antibody test

in sera from cases of human cysticercosis.

In the agar-gel precipitin test when undiluted homologous antiserum was placed in the center well and undiluted antigen on either side of it in diagonal templates, one to two bands of precipitate were observed. This variation in the number of bands may have been due to the presence of one to two reactants in the antigen-antibody system. According to Oudin (1952), only one precipitin band is formed when a single antigen-antibody system is present in a given mixture of antigen-antibody. One to several bands in agar-gel systems are not uncommon in tests involving helminth antigens. Kagan and Bargai (1956) reported three bands in sera from rabbits infected with trichinosis; Kagan and Norman (1961) showed numerous bands in a system involving Echinococcus antigens; and Duwe (1967) reported one to numerous bands in sera from rabbits immunized against Moniezia expansa.

In those tests in which antiserum was diluted and antigen undiluted, low titers were observed, whereas, when the procedure was reversed and the antigen was diluted, higher titers were obtained. Kagan and Norman (1961) suggested that the antibody concentration is the weakest component when used in parasite antigen-antibody systems and that titration of antigen gave the highest titers. A slight

increase in the antibody titer was also observed in the present report when the position of antiserum to antigen was changed in the diagonal templates.

It is of interest to note that cross reactions were not obtained between Echinococcus granulosus and Taenia saginata antisera and Cysticercus fasciolaris antigen. Cross reactions between these species would be expected since these organisms belong to the order Cyclophyllidea and fall within the family Taeniidae. Maddison, Whittle, and Elsdon-Dew (1961) observed cross reactions between Taenia solium antigen and a weak reaction between T. solium antisera and hydatid antigen. The failure to obtain cross reactions between T. saginata and Echinococcus granulosus antisera and Cysticercus fasciolaris antigen might be explained on the basis of host differences between all of these species. T. saginata infects cattle and man while E. granulosus utilizes a variety of intermediate hosts such as swine, deer, and man; whereas, C. fasciolaris is a parasite of rats and cats. Kagan (1967) suggested that cross-reactivity between parasites is possible if they share common hosts and are ecologically related.

In the heterologous system in which C. fasciolaris antigen was placed in the center well and T. crassiceps

antiserum in the peripheral wells, two continuous bands of precipitate were observed. It appears that there were two specific reactants present in this antigen-antibody system. By shifting the position and placing T. crassiceps antiserum in the peripheral wells, only one band was observed. Therefore, the number of bands in this system may probably be due to the position of antigen to antiserum. Moreover, when C. fasciolaris antiserum was placed in the peripheral wells and homologous C. fasciolaris antigen in the center well, a continuous circular band was observed. The circular shape of the precipitin band may have been due to the distance between the peripheral wells and an excess of antibody.

In those tests between C. fasciolaris antigen and Taenia crassiceps antiserum, reactions of identity were observed as a continuous band or loop of precipitate. It is highly probable that the antigens of these two species are common and closely related in spite of the fact that T. crassiceps is an adult form and Cysticercus fasciolaris is a larval form. Furthermore, they appear to have a close phylogenetic relationship in that they have rodent intermediate hosts and are ecologically similar. Wilhelmi (1940) analyzed precipitin tests involving various stages in the life cycle of trematodes and cestodes and observed that

larval and adult antigens are not serologically distinct. He reported that the precipitin titers of the adult and larvae were practically the same. Kagan and Norman (1963) reported some reactions of identity in the agar-gel precipitin tests using various antisera prepared against the life cycle stages of Schistosoma mansoni. Kagan (1957) also reported cross reactions of the nematodes, Ascaris lumbricoides var. suum with Toxocara canis and Toxocara cati.

In the immunoelectrophoresis test, two precipitin arcs appeared to be similar in both the homologous and heterologous systems. In general, these results appear to support the observations on the patterns obtained in the agar-gel precipitin test.

CHAPTER VI

SUMMARY AND CONCLUSION

Serological studies on Cysticercus fasciolaris were were made using the micro-precipitin, agar-gel precipitin, immunoelectrophoresis, and indirect hemagglutination tests. The indirect hemagglutination test was found to be the most sensitive, the agar-gel precipitin test sensitive to a lesser degree, and the micro-precipitin test the least sensitive.

In the agar-gel precipitin tests, one to two bands of precipitate were observed between Cysticercus fasciolaris antiserum and antigen. The presence of these bands may be due to one or two reactants in the antigen-antibody system. The highest antibody titers were observed with undiluted antisera and diluted antigen which indicated that the antiserum concentration may be critical.

Cross reactions were not observed between Echinococcus granulosus and Taenia saginata antisera to C. fasciolaris antigen, possibly due to differences in ecology and specific

host requirements. Cross reactions of identity were demonstrated between T. crassiceps antiserum and C. fasciolaris antigen. It was suggested that a close phylogenetic relationship and common antigens between T. crassiceps and C. fasciolaris may serve as a basis for these findings.

Two precipitin arcs appeared to be shared between C. fasciolaris antigen and T. crassiceps antiserum in the immunoelectrophoresis tests. These observations were found to support results obtained in the agar-gel precipitin test.

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